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DEPARTMENT OF THE ARMY
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#17

Contribution to the mode of transmission of Babesia canis by ticks.

by F. Regendanz and E. Reichenow.

Zentralblatt f. Bakt. I. Orig. 124: 471-478 (1933).

Lounsbourg was the first to succeed in the experimental transmission of Babesia canis through the agency of ticks. He showed that Haemophysalis leachi transmits canine piroplasmosis in South Africa, making the important discovery that the tick's infection is inherited by its progeny and that the next generation only becomes infectious in the imago stage, while its larvae and nymphs do not transmit the parasite. Nuttall was then able to confirm that this tick species is the vector of Babesia canis; he succeeded in infecting a dog in England with this type of tick sent from South Africa. In France, Nocard and Motas, as early as 1902 had suspected another tick, Dermacentor reticulatus, as carrier of canine piroplasmosis; they were unable, however, to present experimental proof thereof since they had made tests only with larvae from an infected mother tick. The proof of the theory that Dermacentor reticulatus carries the disease in France was presented in 1919 by Brumpt. He established that in this tick species the infection is also inherited. Only the adult ticks of the new generation, not the larvae and nymphs, were said to be infectious. He further showed that larvae or nymphs of non-infected mother ticks, infused with piroplasmic blood, do not carry the infection to the adult stage of these ticks, thus negating infection from one stage to another of the same generation. In addition, Brumpt and Larrousse noted that Dermacentor venustus, tick not indigenous to the Old World, also may transmit canine piroplasmosis by passing the infection to the next generation (imagoes). Christophers had discovered earlier that in India another tick species, Rhipicephalus sanguineus, is the vector of Babesia canis. Here the disease is transferred to dogs not only by the adult progeny of infected ticks, but already by the nymphs. Whether this is true also of larvae cannot be deduced with certainty from his tests. Christophers also believes on the basis of his observations of the parasite's development, even though he was unable to so demonstrate experimentally, that a tick becomes infectious also when it has received piroplasmic blood during its nymphal stage. These test results are contradicted by those of Brumpt, who made further investigations with Rhipicephalus sanguineus in Tunis (1919). In contrast to Christophers, he found that although Babesia canis is indeed inherited by this tick, only the imagoes of the following generation are able to transmit the infection to dogs, and that ticks receiving piroplasmic blood in the nymphal stage are not infectious as imagoes at the next feeding. For the sake of completeness it should be mentioned here that James succeeded in infecting dogs in England with Rhipicephalus sanguineus imported from India, and Lenyon followed suit with ticks of the same species from Aleppo. Kleschulz and Mawo-Koenoe (1931), on the other hand, reported that they were unable to transmit a laboratory strain of B. canis through the agency of Rhipicephalus sanguineus.

We have conducted numerous transmissive experiments in the last few years which were aimed primarily at the clarification of the development of Babesia canis in the carrier. We have reported on their results elsewhere (Arch. f. Protistenkunde, 1932). Since the transmissive experiments led to diverse conclusions that complement the experiences listed above in several aspects, they will be summarized here.

Our initial tests were aimed at the transmission of canine piroplasmosis through the tick Rhipicephalus sanguineus. The ticks utilized came from Java (1), the Piroplasma strain from the Pasteur Institute at Paris, made available to our institute in 1925. Since then it had been maintained on dogs, partly here and partly at the I.G.-Farben Works at Elberfeld, without the interpolation of a tick passage. The strain is fairly virulent, dogs without spleens as a rule succumb to the infection 8-10 days (on the average) after super-inoculation of piroplasmic blood, while the infection often takes a milder course in other dogs with spleens, but always accompanied by copious parasites in the blood. In young dogs with spleens the infection is more severe, frequently with a fatal outcome. We used primarily splenectomized dogs, since infections could not be overlooked under these circumstances.

Test series I.

A spleenless dog was infested with a large number of male and female ticks; at the same time the animal was injected with piroplasmic dog blood. Four days later the first parasites appeared in its blood, the infection progressed rapidly and the animal died on the 8th day after injection. Part of the female ticks had already fallen off in a saturated state. Another splenectomized dog was similarly subjected to ticks and an injection of piroplasmic blood on the 3rd day. The course of infection was identical to that of the first dog, a large number of ticks satieted with piroplasmic blood was obtained. These were then maintained singly in jars at a humid temperature of 23-25°C. Production of eggs began after approximately 5 days. The escaping larvae and, later, the nymphs were attached to guinea pigs for feeding, being maintained at the indicated temperature in the meantime; the same was done with the imagoes. The following experiments were conducted with this material:

a) An emulsion in Ringer solution was produced from part of the egg crop of one tick (about 250 eggs) and injected intraperitoneally into a splenectomized dog. The dog remained healthy. It was injected with piroplasmic blood after 3 months and died of the resulting infection.

b) An emulsion of about 500 eggs of another tick intraperitoneally into another splenectomized dog. The animal stayed healthy.

c) Emulsion of larvae of another tick (several hundred) intraperitoneally into another spleenless dog: No infection.

d) A dog with spleen and one without were infested with a large number of larvae from other ticks of this group. Part of these larvae

fell off satiated after a few days: No infection.

e) An emulsion of 4 nymphs emanating from still another tick was injected into a splenectomized dog: No infection, not even after splenectomy. Seven weeks later the dog was inoculated with piroplasmic blood and died of the infection.

f) Adult ticks coming from different mother ticks were attached to dogs. One splenectomized dog received 3 male and 1 female ticks, another spleenless dog got male and female ticks (25 and 23); in addition, 4 dogs with spleens (of which 2 were later splenectomized) received 20 m, 27 f; 22 m, 30 f; 8 m, 11 f; 50 m, 50 f ticks. Piroplasmae could not be found in any of these dogs.

Test series II.

Approximately 60 adult ticks were attached to a dog. On the following day it received an injection of piroplasmic blood. Five days later Piroplasmae appeared in its blood, becoming somewhat more numerous in the next few days. The satiated female ticks fell off, the last one 7 days after the appearance of Piroplasmae in the blood of this dog. The ticks were stored as in test I. The following experiments were made with the material obtained from these ticks:

a) A splenectomized dog was injected with the emulsion of the entire egg crop of one tick: The animal was not infected.

b) Different dogs received injections of egg emulsions of the egg deposits of several ticks: No infection resulted.

c) A dog was infested with 101 female and 106 male ticks from 5 different mother ticks. 80 ticks dropped off after saturation: The dog was not infected.

d) 23 ticks from 2 other mother ticks fed on another dog. Again, infection with piroplasma failed.

After these tests, as well as those of Nieschulz which are already known, had established that no further development of the Babesia canis strain used by us takes place in Rhipicephalus sanguineus, and that it is not inherited by the progeny even if the ticks and their progeny are maintained at higher temperatures, the question arose whether this strain is transmissible at all through this tick species, if perhaps a special race of Babesia canis may be involved here, or whether the utilized strain had lost the ability to develop in the tick owing to its maintenance for years solely on dogs, without the interpolation of tick passages (2). It is true that such observations do not exist for Babesia, but they have been made in connection with other parasitic protozoa, the pathogenic Trypanosomes. The earlier observations of Leichenow and more recent ones by Duke of Trypanosoma gambiense show that the development of this parasite in its natural carrier, Glossina palpalis, is absent or rudimentary if the

Trypanosomes remain in the human or in animal passages without the interpolation of a Glossina passage.

We had another strain from a tick passage (Dermacentor reticulatus) with which to clarify this question (3). Infected adult ticks of this species were attached to a non-splenectomized dog. Piroplasmae were present in its blood after 7 days, the infection increased rapidly and the animal succumbed to the infection 10 days after being subjected to these ticks. We infected another dog from this one; Babesiae were found in its blood 3 days later. Next we attached a number of ticks of the species Rhipicephalus sanguineus to it. Seven days later it died of the infection. Five saturated female ticks separated from the dead dog; the others, not yet satiated, were attached to another dog which had been infected with the blood of the second dog a few days earlier and which now showed severe piroplasmosis. Of the ticks fed on it, 2 re-attached themselves and fell off on the third day. Thus the ticks of this species had also absorbed copious quantities of Babesiae. Repeated transmissive tests were made with the progeny of these 7 ticks, this time only with the nymphs and imagoes issuing from them, after these ticks had been maintained at a higher temperature (22-23°C) throughout their entire development.

Test series III.

A splenectomized dog was infested with about 100 nymphs issuing from these 6 ticks. They attached themselves well and a large part separated from the dog 5-7 days later. The dog was not infected. In the last test with Rhipicephalus sanguineus the imagoes of this generation were attached to a splenectomized dog, a total of 100 female and male specimens. 32 female ticks dropped off satiated 7-12 days later. The dog was not infected.

It was shown thus that Rhipicephalus sanguineus does not even transmit a strain of Babesia canis which shortly before had been transferred to the dog by ticks; while the same strain is very easily transmitted by Dermacentor reticulatus, as will be shown below. Since other investigators (Christophers, Brumpt, Wenyon, James) have established that Rhipicephalus sanguineus is the vector of canine piroplasmosis in India and the Mediterranean area, this contradiction with our test results may be explained only by the assumption that there are races or varieties of Babesia canis which cannot be differentiated morphologically in the dog's blood, and which deviate only by developing in different tick species.

Test series IV.

The following tests were designed to establish the stage in which the progeny of Dermacentor reticulatus becomes infectious, if imagoes have absorbed piroplasmic blood. As evidenced by the test described above, adult ticks of this species had transmitted Babesia canis to a dog. We had attached 12 female and 12 male specimens in this experiment. After 7 days, the first Babesiae were found in the blood of this dog, multiplying

rapidly in the next few days, so that the dog died 9 days after being subjected to the ticks. Six ticks dropped off, and 4 others, not yet fully saturated, were attached to another dog that had been inoculated with the blood of the first dog on the previous day. Parasited appeared in its blood two days after the attachment of ticks, reproducing rapidly and causing the death of this non-splenectomized dog after an additional 7 days. Of the last 4 ticks, 3 dropped off after 3-4 days, the last tick after 8 days of feeding, so that all had absorbed copious quantities of parasites with the blood. The ticks were stored singly in jars at a humid average temperature of 23°C. The following experiments were made with the progeny of these ticks:

a) A splenectomized dog was inoculated with the emulsion of part of the egg crop (about 500 eggs) of the tick that had fed the longest on the second dog and which, as shown by microscopic examination, was infected with Piroplasmae (see our report on the development of Babesia canis in the tick). Since no Babesiae were found in the dog's blood, it received an injection of the entire egg deposit of another tick. This action caused tick paralysis with the typical symptoms recently described in detail by Regendanz and Reichenow in connection with tick paralysis produced by the eggs of Rhipicephalus sanguineus. The animal recovered from the paralysis within a few days. No Piroplasmae were found in its blood.

b) Another splenectomized dog was injected with an emulsion of more than 100 larvae originating with 5 different infected ticks. Again the infection failed.

c) Numerous larvae were attached to a splenectomized dog, of which three fell off satiated after 4 days. The dog was not infected. These larvae belonged to another series (see IV f, V).

d) Later about 20 nymphs from 4 different infected ticks were attached to another splenectomized dog. Of these, 8 dropped from the dog after a few days. Seven days after the attachment its blood revealed Babesiae; they multiplied rapidly and the animal died after 2 additional days.

e) The last experiment was repeated immediately by attaching about 40 nymphs to another spleenless dog, all emanating from another tick. Of these, 12 specimens dropped off saturated after a few days. Six days after the attachment of nymphs this dog also showed Babesiae in the blood, increasing rapidly and causing the death of this animal 9 days after the attachment of nymphs.

f) In addition, nymphs from other mother ticks were fed on splenectomized dogs in two tests; the nymphs came from ticks that had lived through the winter after absorbing piroplasmic blood (see below). These nymphs were reluctant to feed on the dog, only one specimen in one test was found to have fed to satiation; thedog involved was not infected.

Thus the tests have revealed, in contrast to the results of Brumpt, that nymphs of Dermacentor reticulatus from ticks fed on dogs infected with Piroplasmae are capable of transmitting piroplasmosis. The result agrees with the findings of Christophers in connection with Rhipicephalus sanguineus, with the reservation that his experiments were conducted in a country where canine piroplasmosis is endemic and sources of error could not be entirely excluded.

Test series V.

Lastly, we determined the percentage of adult Dermacentor reticulatus being infected.

a) A spleenless dog was subjected to 3 male and 6 female adult ticks descended from infected mother ticks. Of these, only one male and one female attached themselves and dropped off after several days. Nine days after attachment the dog's blood evidenced Piroplasmae, increasing rapidly and causing its death after 3 additional days.

b) Another splenectomized dog was subjected to 3 male and 3 female ticks as in test V a. Of these, only one female remained attached, dropping off after 10 days. The first Piroplasmae appeared in the dog's blood after 8 days; they multiplied in the following days and caused the animal's death 16 days after attachment.

c) We also succeeded in infecting a young, non-splenectomized dog with piroplasmosis by attaching 4 female and 3 male ticks from the same series as in tests V a and V b. In this case the parasites appeared in the blood 7 days after subjection to ticks. The animal died after 5 additional days.

In evaluating the results of this test series it must be borne in mind that not only females are involved as vectors of the infection (easily determined by means of satiated specimens), but undoubtedly the males also, although this cannot be established in the same manner. For our findings reported elsewhere have shown that the development of Babesiae in the salivary glands occurs in the males in the same manner as in the females. Still, it follows from the small number of ticks used in each infective test that nearly all progeny of a mother tick in possession of Babesial blood become infectious. The successful infections carried out by Brumpt and Larrousse with 2 females and 1 male of Dermacentor venustus tend to support this opinion. In could be concluded from our negative result with one nymph (test IV f) that the infection of the progeny is not 100%; however, microscopic examination of the salivary glands revealed that while not all nymphs are infectious, all imagoes definitely are.

In the case of Dermacentor reticulatus, the depositing of eggs begins in spring and summer, 2-3 days after abandoning the host animal. In our attempts to breed this tick species the laying of eggs started considerably later, in some cases after 60 days, in others still later or not at all. The ticks of this series had absorbed piroplasmic blood in

September and were maintained at a humid temperature of about 23-25°C. When in the following year ticks that had fed in September had not started to lay eggs 8 days after dropping off, we assumed that this species of tick might benefit from the interpolation of the winter season. We therefore stored the ticks for 2 months in a cool room in which the temperature in the months of November and December dropped to about +9°C. At the end of December these ticks were returned to a warmer temperature (about 23°C), which was followed by the deposit of eggs in large amounts a few days later. The ticks' further development proceeded normally.

Subsequently, ticks of this series were used for a few transmissive tests, the first of which were conducted with nymphs and have already been listed under IV f. Adult ticks of this series were attached to a young, non-splenectomized dog in February, a total of 12 females and 10 males. The dog's blood showed Piroplasmae after 7 days, which increased in the next few days and then decreased a few days later. In was demonstrated thereby that the Babesial infection of the tick and the eggs, respectively, had not been eliminated by wintering for the indicated period and temperatures dropping to +9°C.

Summary.

The transmission of canine piroplasmosis through the agency of the tick Rhipicephalus sanguineus failed.

Two strains of Piroplasma were used in these tests, one having been passed on for years from dog to dog, while the other had just been transmitted to the dog by Dermacentor reticulatus.

On the other hand, transmission of Babesia canis by Dermacentor reticulatus always succeeded without difficulty.

Since other investigators had previously succeeded in transmitting canine piroplasmosis by means of Rhipicephalus sanguineus, it is assumed that there are races of Babesia canis which differ only in their suitability to different vectors (tick species).

The Babesial infection of Dermacentor reticulatus is inherited by its progeny. We did not succeed in producing piroplasmosis in the dog by injecting infected eggs and larvae or by the attachment of larvae. On the other hand, nymphs do transmit canine piroplasmosis during feeding, just as the adult ticks.

Dermacentor reticulatus passes the Babesial infection to almost the entire progeny. The male ticks are also capable of infecting, since their salivary glands are infected with Babesiae in the same manner as the females.

The Babesial infection is not extinguished by the wintering of female ticks (cooling to +9°C) after absorption of piroplasmic blood and prior to egg laying.

NOTES.

(1) This tick strain was made available to us by Dr. Nieschulz-Utrecht. It is the same material as used by Nieschulz and our tests were conducted at approximately the same time.

(2) The latter is also being assumed by Nieschulz as an answer to his negative results.

(3) We received it from Dr. Brumpt-Paris, who sent us ticks of this species infected with Babesia canis.